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Title: *Theta+N liquid transfer system for polymer (including DNA and RNA) synthesis reactions conducted in a 96 well plate*

Author: Fernando Andrade, M.S., CSO & CEO of Radegen Bio, Clint, TX 79836, www.radegenbio.com

About Radegen Bio: Radegen Bio is a synthetic biology biotech company founded by Fernando Andrade M.S. that focuses on providing customers with bona fide synthetic DNA, RNA, protein, glycoprotein molecular preparation and synthetic molecular biology workflow solutions. Radegen Bio is passionate about what the future holds for humanity in light of recent innovations in the life sciences that hold the key to advances in biomedical sciences, energy production, material sciences, computer science, chemistry, environmental science and engineering. Although we are still at the inception of our mission, I hope that the work I produce as CSO of Radegen Bio has a significant positive impact on the molecular biology community by providing synthetic central dogma macromolecules that can be used in various applications and are provided in small to medium scale. These affordable solutions for quick molecular prototyping using RUO products provide industry the power of optimizing custom solutions. Our solutions provide academics the same production scale as big pharma to go from idea to publication in a matter of months instead of years. These solutions employ **proprietary** and **open-source intellectual property** to produce powerful enzymatic solutions using technologies discovered by the molecular biology community over the past 70 years and now developed by Radegen Bio's synthetic biology framework.

Impact: Synthetic DNA (synDNA) has the potential of revolutionizing research and development efforts in the life science industrial and academic community. The primary benefit being that the rate of discovery is accelerated due to the availability of readily available, high quality genetic constructs and synDNA accessories. Synthetic genetic constructs are usually purchased either whole or in sub fragments and typically used to create a synthetically recombineered genes or larger genomic molecule. Accessory DNA including qPCR controls for multiplex reactions is commonly purchased in a synthetic DNA format because it automatically provides equimolar template copy number counts due to the ability to design multiple templates on a synthetic DNA molecule. The applications for synthetic DNA are vast and the seemingly infinite degree of positive impact that solutions for problems associated with global sustainability and biomedical research are very promising. In order to meet these demands, more widely available and affordable solutions need to be developed. It is Radegen Bio's purpose to implement the currently developed *in silico* solutions in our technology portfolio to the Texas, USA scientific community with quick turnaround times and provide research across the country with innovative solutions based on Radegen' Bio synthetic biology framework.

The Creative Concept: As a graduate student my research focus was the study of surface associated bacterial communities and addressed ecological questions to characterize factors that may mediate attachment to a surface and intracellular associations. Since my job was essentially to count microbes attached to a plastic coupon my primary research tool was qPCR. As you can imagine this involved a lot of pipetting between plates containing qPCR master mix and gDNA. Defined variability between duplicate samples treated with multiple variables facilitated setting up master wells strategically positions so that equally spaced pipet tips on a multiple channel pipettor could aspirate solution from the master plate to the reaction plate. Since then, my area of expertise has evolved within the last few years to synthetic biology and I set out to develop a *de novo* nucleic acid synthesis process and the experience acquired in graduate school inspired a novel liquid transferring system that accelerates the output of commercially available automated robotic liquid handling robots over 4x.

When I set out to develop a new system, one the desired characteristics was that it be based on an open-source platform to provide accessibility to developing countries in dire need of an affordable solution for molecular testing, especially in the face of the COVID pandemic. A method using equipment already available in a common laboratory would be ideal since the implementation would be easy. DNA is a polymer composed of 4 monomers so the pipetting needs of a 96 well plate require a system for adding one of 4 monomers into a well for a stepwise coupling reaction. Each sequential well may requires a different nucleotide monomer so the use of a multichannel pipettor is prohibited because it would require a new plate serialized with nucleotides in the same order needed in the reaction plate to polymerize the distinct monomer required in the DNA sequence being elaborated in the well. This would require that stacks of plates be prepared ahead of time, a process that would add time to the process. The development of a monomer

addition process where multiple wells can be filled with one dispensing step is needed to reduce the time required to commence a coupling reaction. The Theta+N liquid handling system provides the option of dispensing liquid into multiple wells using a multichannel pipettor that is either handheld or processed by liquid handling robot.

There 4 monomers in a DNA polymer, there are 256 possible 4 bp combinations for each position in a DNA sequence. Reaction master mix containing one of each monomer are distributed in three 348 well plates in a sequential order with one nucleotide per well with a sequential order of one 4 bp combination distributed in 4 sequential wells in a row. This strategy allows for the accurate liquid transfer into 4 sequential wells in a row at the same time with one dispensing event, providing the reagents for extending a polymer by one monomer by a polymerization reaction that occurs in a reaction well. A synthesis involves adding reaction buffer to each well one at a time requires multiple additions and removals of reaction buffers making the total time of a reaction cycle reaching 7.4 min. A reaction cycle using Radegen Bio's chemistry in the absence of the Theta+N liquid transfer system contains multiple steps each containing a directive between 15 sec – 6 mins that add buffers required for synthesis. Radegen Bio's synthesis chemistry is currently benchmarked at producing 100-unit fragments with a total yield of polymerized monomer of 9600 units broken into 96 fragments, each produced in a single well, polymers with distinct monomer sequences and a total synthesis time of 12 hours. The implementation of Radegen Bio's Theta+N system cuts the time for a synthesis cycle, producing the same products as described above to 2.9 mins. At 4.8 hours the total daily yield is 480 fragments, 48,000 polymerized monomers per day at 100 units per fragments. The productivity of one liquid handling robot increases over 4x approximately every 24 hours period of sequential synthesis. There is no doubt that Radegen Bio's liquid transfer system revolutionizes the synthetic DNA discipline by providing an open source, licensable polymer synthesis solution that is available to both academics and commercial industry. This is a great accomplishment that deserves to be shared with the world immediately and is a case study of how valuable a research-intensive graduate school education can incept simple ideas that result in a revolutionary system first implemented for qPCR and its adoption in nucleic acid synthesis resulted in a complete synthesis solution, including ssDNA synthesis and dsDNA assembly using a common platform with efficiencies four times greater than legacy standards. The power of exponents can also be limiting, and this is one of those cases. Modifications to this concept that aim at modifying throughput by either increasing the number of liquid handling transfers per transfer event from a reagent reservoir to a reaction plate or the number of subunits in a monomer either increase total synthesis time or result in reagent reservoir containers in numbers that cannot be efficiently processed.

Rational for licensing via the Creative Commons architecture:
Radegen Bio wishes to contribute to the synthetic biology community and developing countries by providing open-source access to this novel creative concept. The lack of any published work within the life sciences industry and manufactures of robotic automated instrumentation demonstrate the uniqueness of such approach by the lack of any previous work describing this concept. Generating a work that implements a process using this concept is prohibited because it would require a variation or derivative of the creative concept be it digital, print, or in handwriting. For example, a commercial entity that produces a variation in the way a microtiter plate is prepared or in liquid transfer because the work would be a variation or derivative of the core concept described in this creative work. Protection is mediated by preventing a variation or derivative work from being created in any media and thus a commercial operation could never implement the use of the creative concept. This creative concept has the potential for use in other synthesis platforms, either under development or pre-existing and since the novel creative concept of 4 simultaneous additions into independent reaction wells that preserve the desired sequence identity of the final molecule is a completely novel process, a work describing a variation or derivative could never have a claim of being a derivative or variation of a different process. The process is also protected by the mechanism described above. Creative commons licensing is a great choice for the synthetic biology community to consider when obtaining intellectual rights protection for any kind of creative work.